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Animal Models to Study Thyroid Hormone Action in Cerebellum

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Abstract Thyroid hormone plays a crucial role in the development and functional maintenance of the central nervous system including the cerebellum. To study the molecular mechanisms of thyroid hormone action, various animal models have been used. These are classified: (1) congenital hypothyroid animals due to thyroid gland dysgenesis or thyroid dyshormonogenesis, (2) thyroid hormone receptor (TR) gene-mutated animals, and (3) thyroid hormone transport or metabolism-modified animals. TR is a ligand-activated transcription factor. In the presence of ligand, it activates transcription of target gene, whereas it represses the transcription without ligand. Thus, phenotype of TR-knockout mouse is different from that of hypothyroid animal (low thyroid hormone level), in which unliganded TR actively represses the transcription. On the other hand, human patient harboring mutant TR expresses different phenotypes depending on the function of mutated TR. To mimic this phenotype, other animal models are generated. In addition, recent human studies have shown that thyroid hormone transporters such as monocarboxylate transporter (MCT) 8 may play an important role in thyroid hormone-mediated brain development. However, MCT8 knockout mouse show different phenotypes from a human patient. This article introduces representative animal models currently used to study various aspects of thyroid hormone, particularly to study the involvement of the thyroid hormone system on the development and functional maintenance of the cerebellum.

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Molecular Mechanisms of Thyroid Hormone Action

Thyroid hormone (L-triiodothyronine, T3; L-tetraiodothyronine, thyroxine, T4) binds to thyroid hormone receptor (TR) and regulates transcription of target genes [1]. TR belongs to steroid/thyroid hormone receptor superfamily that is a ligand-activated transcription factor. TR genes are encoded in two genetic loci, termed as α and β, which are located at chromosome 17 and 3 in human, and 11 and 14 in mouse, respectively [2]. Each locus produces at least two proteins, which are termed as TRα1 and α2 (or c-erbAα2), and TRβ1, TRβ2 and TRβ3. TR forms homodimer or heterodimer with retinoid X receptor (RXR) and binds to thyroid hormone response element (TRE) located at the promoter region of target genes. TR binds to TRE regardless of the presence of T3 and regulates transcription in a ligand-dependent manner. In the presence of T3, it recruits protein complexes called coactivators to activate transcription, whereas in the absence of T3 it recruits corepressor complexes to repress transcription. It should be noted that, although TRα2 can binds to TRE, T3 cannot bind to it. TRα2 may act as an endogenous inhibitor for other TRs. Because of this bi-directional function of TR, the phenotype of TR gene knockout mouse is different from that of hypothyroid (thyroid hormone deficient) animals [3]. Thus, TR-gene knockout and hypothyroid animal models that are induced by thyroid dysgenesis or dyshormonogenesis are equally important to understand the roles of the thyroid hormone system in the brain.

Animal Models for Thyroid Gland Dysgenesis or Thyroid Dyshormonogenesis

There are several genes involved in congenital hypothyroidism due to thyroid gland dysgenesis or thyroid dyshormonogenesis [4]. The former includes thyroid transcription factors (TTF)-1, TTF-2, Pax8, and thyrotropin (TSH) receptor, and the latter includes thyroid peroxidase (TPO), dual oxidase (Duox), thyroglobulin (Tg), sodium-iodide symporter, and pendrin. Several representative animal models to study the effect of congenital hypothyroidism induced by such gene defect or malfunction are listed in Table 1.

Animal Models for Thyroid Gland Dysgenesis

The commonly used animal model for thyroid gland dysgenesis is Pax8 knockout mouse [5]. Pax8 is essential for thyroid follicular cell differentiation, and thus, its knockout mouse shows severe hypothyroidism. Morphological development and gene expression in the cerebellum is greatly affected in this mouse [6]. Since abnormal organogenesis induced by Pax8 knockout seems to be confined in the thyroid, this mouse model can be an ideal model to study the molecular mechanisms of thyroid hormone action in the brain.

On the other hand, TSH also plays a critical role for the development and function of the thyroid gland. A natural mutant mouse of TSH receptor, *hyt/hyt* mouse, harbors a point mutation of C to T at nucleotide position 1666, which replaces Pro with Leu at position 556 in transmembrane domain IV of the TSH receptor [7]. Mutation of this region may inhibit translocation of TSH-receptor from cytoplasm to cell membrane, resulting in low TSH binding [8]. This mouse shows significantly delayed somatic and behavioral development, which is a typical phenotype for congenital hypothyroidism [7]. However, their general growth returns to normal as they grow [9], and the cerebellar phenotype is limited. Thus, the magnitude of hypothyroidism is mild. However, more than 20 families harboring TSH receptor mutation has been reported, some of which harbor mutation of transmembrane domain IV [8]. Thus, this mouse can be a good model to study the pathophysiology of such disease.

There are additional animal models of thyroid dysgenesis, such as TTF-1 or TTF-2 knockout mice, which show disrupted thyroid genesis accompanied with additional organ defect. To study the molecular mechanisms of thyroid action in brain, these mouse models may not be ideal because of such additional defects.

Animal Models for Thyroid Dyshormonogenesis

There are several critical steps in thyroid hormone synthetic pathway. Disruption of such steps may induce thyroid dyshormonogenesis. For example, TPO regulates oxidation and organification of iodide and coupling of iodotyrosine to iodothyronine. Abnormality of any such steps induces a decrease in thyroid hormone synthesis [10].

Drug-Induced Hypothyroidism

Hypothyroid animal models induced by administration of anti-thyroid drug such as propylthiouracil (PTU) and methimazole (methylmercaptoimidazole, MMI) have been commonly used to study thyroid hormone action in the cerebellum [11]. These drugs inhibit synthesis of thyroid hormone by inhibiting TPO activity. Since the TR knockout mouse model may not always be a suitable model to study thyroid hormone (or TR) action, these are still useful animal models [12]. It should be noted that the phenotype of drug-induced hypothyroidism differs between adult and developing animal, since thyroid hormone affects growth and

Table 1 Mutant animals showing congenital hypothyroid phenotype

Species	Name	References	Etiology	Representative phenotypes
Thyroid gland dysgenesis				
Mouse	*Pax8*$^{-/-}$	[5]	Pax8 gene knockout	Severe thyroid gland dysgenesis
Mouse	*hyt/hyt*	[7]	TSH receptor mutation	Relatively milder hypothyroid phenotype
Thyroid dyshormonogenesis				
Mouse/rat	PTU or MMI treated	[11]	Inhibition of TPO activity	Hypothyroidism at various severity
Mouse	*tpo*	[15]	Mutation of TPO gene	Severe hypothyroidism with goiter; brain phenotype not known
Mouse	*thyd*	[16]	Mutation of dual oxidase 2 (Duox2) gene	Severe hypothyroidism with goiter; hearing impairment
Mouse	*cog/cog*	[18]	Thyroglobulin gene mutation	Large goiter but mild hypothyroid phenotype
Rat	*rdw*	[19]	Thyroglobulin gene mutation	Severe hypothyroidism with thyroid gland atrophy Various brain phenotypes

differentiation of many organs only during a limited critical period during development. In the rodent cerebellum, the effect of the anti-thyroid drugs is greatest during the first two postnatal weeks, which is a period when dendritic growth of Purkinje cell, proliferation and migration of granule cells, and synaptogenesis of cerebellar neurons are active [13]. It should be also noted that the drug response is weaker in mouse than in rat, and additional drug such as sodium perchlorate is sometimes required to render them hypothyroid [14].

Mutant Animals Showing Thyroid Dyshormonogenesis

Thyroid dyshormonogenesis has been shown to result from a number of different genes. There are many mutant mouse models harboring mutation of genes responsible for thyroid hormone synthetic pathway [14]. To study the role of thyroid hormone in the brain, however, abnormal phenotype directly induced by such mutation should be confined within the thyroid gland. Animal models that may fit for such criteria are those harboring disruption of TPO activity [15–17] or Tg mutation [18, 19].

Takabayashi et al. have reported a natural dwarf mutant mice, named *tpo*, showing a severe hypothyroid phenotype (a distinct growth retardation with short life span) with reduced T3 and T4, and elevated TSH plasma levels [15]. Although brain phenotype has not yet been studied, *tpo* mouse is potentially a good model to study thyroid hormone action in brain. Another mouse model with disrupted TPO activity is *thyd* mouse, in which gene encoding dual oxidase 2 (Duox2) gene is mutated [16]. Since Duox generates the hydrogen peroxide needed by TPO for organification of iodide, this mutation causes abnormal TPO activity that induces a severe hypothyroidism. Although its cerebellar phenotype has not yet been examined, this mouse shows a hearing impairment that is a typical neurological phenotype of congenital hypothyroidism. Thus, this mouse is also potentially a good model to study thyroid hormone action in brain. In addition to Duox2 gene mutation, a recent report has shown that mutation of Duox maturation factor 2 (Duoxa2), which is required to express Duox2 enzymatic activity, causes congenital hypothyroidism in human [17]. Although the animal models harboring Duoxa2 mutation have not yet been generated, such animal model could be an interesting animal model to study.

Another group of animals showing congenital hypothyroid phenotype are those harboring mutations of the Tg gene. Thyroid hormone is made within this molecule. The Tg protein consists of 2,749 and 2,766 amino acids in human and mouse, respectively, and a number of mutations have been identified from many species [20]. In mouse, a congenital hypothyroid mice strain with a large goiter induced by mutation of Tg gene is known as *cog/cog* mouse [18]. Although a detailed analysis has not yet been done, its cerebellar weight is significantly less than those of normal mice [21]. However, hypomyelination is restricted in the cerebrum and its general growth returns to normal with the advance of age, indicating that its hypothyroidism is rather mild. On the other hand, a rat congenital hypothyroid strain named *rdw* rat shows severe hypothyroidism with no goiter formation [19]. Our preliminary results have shown the abnormal motor coordination with decreased dendritic arborization of Purkinje cells, indicating that this rat can be an ideal model to study the effect of hypothyroidism in cerebellum (manuscript in preparation).

Animal Models for Thyroid Hormone Receptor Gene Mutation

A number of TR gene-modified mice have been generated. These are roughly classified as: (1) TR gene knockout mice, and (2) TR gene knockin mice. In addition, there are several mouse models, which overexpress TR in a tissue-specific manner such as in the pituitary and heart. However, to study thyroid hormone or TR action in the brain, these models are not suitable and thus will not be discussed in this article. Compared to TRβ proteins, TRα proteins are more abundantly expressed in the brain. Thus, a greater alteration of brain phenotype is observed by mutation of the TRα gene.

TR Knockout Mice

As discussed above, TR has bi-directional actions of transcriptional regulation of target genes. Thus, the effect of TR deletion is different from those of low thyroid hormone level. To study the role of TR on organ development and function, TR knockout mice are essential. Figure 1 shows TR protein isoforms that can be generated from the TR loci. There are two TR genetic loci termed as α and β, each of which produces several functional TRs as a result of alternative splicing and/or differential promoter usage. Furthermore, some introns have a weak promoter activity such as intron 7 of the TRα gene. Thus, deletion of upstream exon may result in the expression of additional TR-related proteins, whose expression is limited under normal condition [22]. So far, at least three additional TR-related proteins may be generated. Such proteins, termed as TRΔα1, TRΔα2, and TRΔβ3, lack N-terminus and DNA-binding domain and cannot bind to TRE. Thus, phenotypes of TR knockout mice may be due to a combination of deletion of a specific TR with overexpression of other TR species. Table 2 shows the list of TR knockout mice. Possible remaining TR proteins in each animal are also indicated.

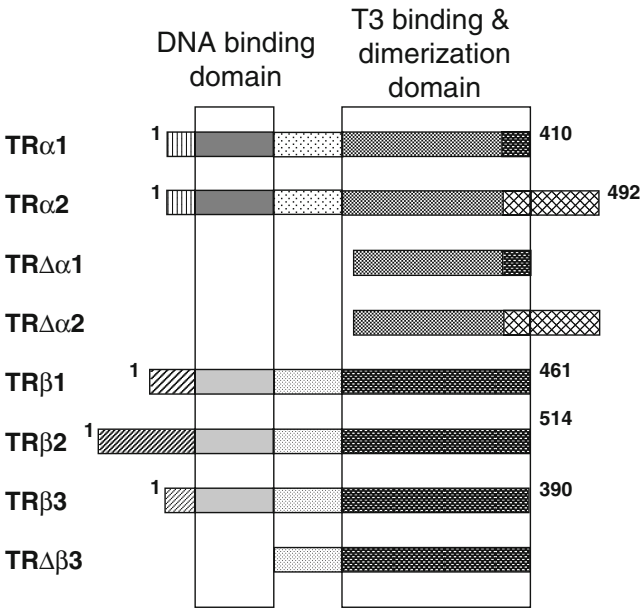

Fig. 1. Thyroid hormone receptor and its related proteins generated from α or β gene locus. Numbers indicate the number of amino acid. *Hatched region with the same pattern* indicates that the amino acid sequence is identical

TRα1-deleted mice show slightly reduced thyroid function with almost normal phenotype except for 20% reduced heart rate, prolonged QRS and QT durations, and 0.5°C lower body temperature [23]. A limited alteration of behavior and neural circuit is also reported [24]. However, their cerebellar phenotype appeared to be normal except for aberrant maturation of astrocytes [25]. More strikingly, deletion of TRα1 prevents structural alteration of cerebellum in hypothyroidism that is induced by MMI and perchlorate treatment [26]. These results indicate that abnormal cerebellar phenotype in a thyroid dyshormonogenesis animal may be due to a dominant-negative action of unliganded TRα proteins. On the other hand, TRα2 knockout mouse shows both hyper- and hypothyroid phenotype in an organ-specific manner [27]. This may be due to elevated expression of TRα1 in this mouse. TRα1 expression in the brain is also elevated, but cerebellar phenotype is not clear. Deletion of both TRα1 and TRα2 also shows only limited phenotype in the cerebellum. However, apart from cerebellar phenotype, existence of TRΔα1 and/or TRΔα2 shows altered phenotype in various organs. When TRα1 and TRα2 are deleted but TRΔα1 and TRΔα2 expressions are not inhibited ($TR\alpha^{-/-}$) [28], their phenotype is more severe than those of mice in which all TRα proteins are deleted ($TR\alpha^{0/0}$) [29, 30]. The greater decrease in plasma thyroid hormone levels, the more severe the impairment of bone and intestine development observed.

More limited brain phenotype is observed in TRβ knockout mice. While TRβ1 is widely expressed including the cerebellum, particularly, in the Purkinje cell [13], the expression of TRβ2 is confined within the pituitary, hypothalamus (TRH neuron), retina, and inner ear. TRβ2 knockout mice show central resistance to thyroid hormone with elevated T3, T4, and TSH levels in serum [31]. Furthermore, this deletion causes a selective loss of M cones in the retina [32]. However, abnormal brain phenotype seems to be confined within the hypothalamus, and any change in cerebellar phenotype has not been reported. In TRβ knockout mouse, on the other hand, it shows aberrant development of auditory function in addition to central hypothyroidism [33]. However, although TRβ is strongly expressed in the Purkinje cell, its deletion does not induce any alteration of thyroid hormone-responsive genes in the cerebellum [34].

In case of TRα and β double knockout, since one receptor cannot substitute the function for the other, their phenotypes are more severe than those of single gene knockout. In $TR\alpha1^{-/-}TR\beta^{-/-}$ mice, delayed general growth and aberrant bone maturation, which are not seen in each single knockout mouse, are observed [35]. In $TR\alpha^{-/-}TR\beta^{-/-}$ mice, aberrant intestinal development, which is seen in $TR\alpha1^{-/-}$, and high T3, T4, and TSH levels, which are seen in $TR\beta^{-/-}$, are observed, both of which are more severe than those of single knockout mice [36]. However, in $TR\alpha^{0/0}TR\beta^{-/-}$ mice, while low body temperature and abnormal auditory function, which are more severe than those of $TR\alpha^{0/0}$ or $TR\beta^{-/-}$, respectively, are seen, aberrant intestinal development is milder than those of $TR\alpha^{-/-}$ $TR\beta^{-/-}$ or $TR\alpha1^{-/-}$ [29]. These results indicate the possible contribution of TRα variants (Δα1 and/or Δα2) in generating differential phenotypes. Alteration of brain development of these double knockout mice has not yet been studied in detail.

TR Knockin Mice

The syndromes of resistance to thyroid hormone (RTH) are characterized as reduced action of thyroid hormone in thyroid hormone target tissue [37]. The majority of cases of RTH is generalized RTH, whereas pituitary RTH is also reported. In addition to elevated serum levels of T3 and T4 levels with non-suppressed TSH, many patients show clinical features related to neurological disorders such as hyperactivity and learning disability. Animal models that mimic the RTH patients are generated (Table 3). The majority of patients harbor a mutation in the TRβ gene. Several knockin mice harboring mutated human TRβ have been generated [38, 39]. Their phenotypes are similar to those seen in human RTH patients such as elevated levels of T3 and T4 with unsuppressed TSH levels in the serum,

Table 2 Thyroid hormone receptor (TR) gene knockout mouse models

Targeted gene	Targeted exon	References	Deleted TRs	Remained TRs	Representative phenotypes
TRα					
TRα1$^{-/-}$	Exon 9	[23–26]	α1, Δα1	α2, Δα2, all β	Normal T3 with slightly reduced T4 level Prolonged QRS and QT durations Prevention of hypothyroid phenotype in the cerebellum
TRα2$^{-/-}$	Exon 10	[27]	α2, Δα2	α1, Δα1, all β	Overexpression of TRα1, inducing both hyperthyroid phenotype (high body temperature, increased hear rate) and hypothyroid phenotype (increased body fat)
TRα$^{-/-}$	Exon 2	[28]	α1, α2	Δα1, Δα2, all β	Aberrant intestine and bone development
TRα $^{0/0}$	Exon 5–intron 7	[29, 30]	All α	All β	Aberrant intestine and bone development, but the phenotype is less severe than those in TRα$^{-/-}$
TRβ					
TRβ2$^{-/-}$	Exon 2	[31, 32]	β2	β1, (β3, Δβ3) all α	Central resistance to thyroid hormone levels Elevated TSH, T3, and T4 Selective loss of M cone in retina
TRβ$^{-/-}$	Exon 3	[33, 34]	All β	All α	Central resistance to thyroid hormone Elevated TSH, T3, and T4 levels Aberrant auditory function development
TRα and *β*					
TRα1$^{-/-}$TRβ$^{-/-}$	See above	[35]	α1, Δα1, all β	α2, Δα2	High T3 and T4 levels due to high TSH Growth retardation; abnormal bone maturation
TRα$^{-/-}$TRβ$^{-/-}$	TRα$^{-/-}$: see above TRβ$^{-/-}$: exon 4–5	[36]	α1, α2, all β	Δα1, Δα2	Aberrant intestine and bone development (more severe than TRα$^{-/-}$) Elevated TSH, T3, and T4 levels (more severe than TRβ$^{-/-}$)
TRα$^{0/0}$TRβ$^{-/-}$	TRα$^{0/0}$: see above TRβ$^{-/-}$: exon 4–5	[29]	All α All β	None	Reduced body temperature and bone maturation (more severe than TRα$^{-/-}$) Aberrant auditory function (more severe than TRβ$^{-/-}$) Aberrant intestine development (milder than TRα$^{-/-}$, or TRα$^{-/-}$TRβ$^{-/-}$)

delayed general growth, and goiter. At least one of these mutations shows an aberrant cerebellar development similar to those seen in congenital hypothyroid animals [39]. This mouse shows decreased arborization of Purkinje cell dendrite with aberrant locomotor activity and decreased expression of thyroid hormone-responsive genes in the cerebellum. In addition, although the RTH patient harboring mutated TRα gene has not yet been identified, knockin mice harboring mutant TRα have also been generated to examine the involvement of unliganded TRα on the development and functional maintenance of the target organ including the cerebellum [40–43]. Compared to TRβ-mutated mice, their neurological phenotype is more severe. This tendency is evident when the phenotypes of mice harboring the same point mutation in TRα and β are compared [41]. Mutant TRα knockin mice show various

Table 3 Mutant TR knockin mice

Targeted gene	Replaced TR	References	Representative phenotypes
TRβ1	PV[a]	[38]	Growth retardation, decrease in thyroid hormone-sensitive gene in various organs Elevated T3, T4 and TSH levels
	D337T	[39]	Abnormal development of cerebellum and hippocampus Elevated T3, T4, and TSH levels
TRα1	PV[a]	[40, 41]	Growth retardation; increase in morality rate Mild elevation of T3, T4, and TSH levels Decrease in cerebellar gene expression
	R384C	[42, 43]	Growth retardation; severe neurological abnormalities including impaired locomotor activity possibly due to impaired cerebellar development

[a] PV are initials of a patient harboring a mutation in exon 10 of TRβ gene, a C-insertion at codon 448, which produces a frameshift of the carboxy-terminal 14 amino acids of TRβ1

aberrant cerebellar developments similar to those seen in congenital hypothyroid animals. These results indicate that TRα may be more involved in regulating cerebellar gene expression than TRβ.

Thyroid Hormone Transport or Metabolism-Modified Animals

Circulating thyroid hormone cannot cross the blood–brain barrier (BBB) without specific transporters [44]. At least two thyroid hormone-specific transporters are expressed in BBB. One is monocarboxylate transporter (MCT) 8, the other is organic anion transporting polypeptide (Oatp) 1c1. Transthyretin (TTR), a major plasma thyroid hormone binding protein particularly in rodents, is also expressed at choroid plexus and has been considered to play an important role in transporting thyroid hormone across the choroid plexus–cerebrospinal fluid barrier. However, thyroid hormone concentration in the brain is not altered in TTR knockout mice, suggesting that TTR is not required for thyroid hormone distribution in the brain [45]. After entering the brain, the thyroid hormone is taken up by astrocyte or tanycyte in which T4 is converted to T3, an active form of thyroid hormone, by type 2 iodothyronine deiodinase (DI) and transferred to neuron or oligodendrocyte [13]. Thyroid hormone is further de-iodinated by type 3 DI, which is mainly expressed in neurons. Modification of these thyroid hormone metabolic pathways may affect greatly the development and function of brain including the cerebellum. Model mice harboring deletion of these genes are summarized in Table 4.

Recently, a novel syndrome that is associated with a severe mental retardation, low muscle tone, spasticity with episodic involuntary movement, absence of speech development, and elevated T3 levels in serum, is reported. These patients harbor mutated MCT8 [46]. Thus, this protein may play a critical role in thyroid hormone-mediated brain development. Recently, MCT8 knockout mice have been generated by two separate groups [47, 48]. Although the patterns of serum thyroid hormone levels are similar to those seen in a human patient, histological examination shows no abnormal development in the brain including the Purkinje cell, which expresses MCT8, despite the low levels of thyroid hormone in the brain [48]. The cause of the discrepancy of phenotype between human and mouse models has not yet been elucidated. Further study may be required to clarify the cause of such discrepancy. In addition to MCT8, Oatp 1c1 may also play an important role in thyroid hormone-mediated brain development. However, until recently, human patient or animal model harboring mutation of Oatp 1c1 has not yet been reported.

A type 2 DI-deleted mouse has been generated [49]. This mouse shows decreased T3 content in most brain regions including the cerebellum, which is as severe as those in hypothyroid mouse brain. However, the change in thyroid hormone-responsive genes in the cerebellum and behavioral alteration such as on the rotarod apparatus is much milder than those in hypothyroid mice [50]. These results indicate that a possible compensate mechanism may play a role to minimize functional abnormalities. Knockout mouse for type 3 DI also has neurological phenotype [51]. Since this enzyme is involved in inactivation of T3, deletion of this gene induces a marked elevated level of T3 during perinatal development, inducing an up-regulation of thyroid hormone-responsive genes such in the cerebellum. However, cerebellar morphological alternation has not yet been studied. Further study is required to examine the involvement of type 3 DI on cerebellar development. It should be noted that there is a type 1 DI that is mainly expressed in the liver. Deletion of type 1 DI does not alter brain development [52].

Animals Showing Similar Cerebellar Phenotype as Hypothyroid Animals

In addition to the animal models discussed herein, there are several additional animal models showing neurological phenotypes similar to those seen in hypothyroid animals.

Table 4 Gene-modified mice harboring deletion of gene involved in thyroid hormone metabolic pathway

Gene	References	Representative phenotypes
Transporters		
MCT8	[47, 48]	Elevated T3 and TSH, and decreased T4 in serum Decreased T3 and T4 levels in brain Much milder neurological phenotype than in patient harboring the same mutation
Deiodinase (DI)		
Type 2 DI	[49, 50]	Decrease in T3 content in brain Mild neurological phenotype, compared with hypothyroid animal
Type 3 DI	[51]	Marked elevation of T3 during perinatal development, inducing up-regulation of cerebellar thyroid hormone-responsive genes

For example, a natural mutant mouse called *staggerer*, which harbors a mutated retinoid receptor-related orphan receptor (ROR) α, shows cerebellar phenotypes similar to those in hypothyroid animal, although their thyroid function is normal. Such similarity is partly induced through the crosstalk between RORα and TR: thyroid hormone regulates RORα gene expression during postnatal cerebellar development [53], and RORα is required for full function of TR-mediated transcription [54]. Since TR and RORα are coexpressed in many neurons including the Purkinje cell, disruption of RORα function may result in the hypothyroid-like phenotype due to such crosstalks.

Another animal model, steroid receptor coactivator-1 (SRC-1) knockout mouse, shows a similar cerebellar phenotype as congenital hypothyroid animals. Since cofactor proteins are required for TR function, and SRC-1 is most abundantly expressed in the cerebellum, deletion SRC-1 gene also induces hypothyroid-like cerebellar phenotype [55]. Indeed, phenotypes of such animals are induced not only by disruption of the thyroid hormone system, but also by other pathways. However, these mice may be also useful to analyze the mechanisms of TR-mediated transcription.

Conclusion

In this article, animal models that can be used to study the role of thyroid hormone and/or TR on brain development have been described. These are classified as: (1) congenital hypothyroid animals due to thyroid gland dysgenesis or thyroid dyshormonogenesis, (2) TR gene mutated animals, and (3) thyroid hormone transport or metabolism-modified animals. Interestingly, not all such animal models show hypothyroid phenotype. While thyroid gland dysgenesis or thyroid dyshormonogenesis animals show a typical congenital hypothyroid brain phenotype, TR knockout animals show relatively mild or no brain phenotype. This may be because TR has bi-directional functions. While it activates transcription of target genes in the presence of the ligand, it actively represses transcription in the absence of the ligand. Thus, hypothyroid phenotype in "low thyroid hormone" animals may be induced through unliganded TR, whereas TR knockout animals show milder phenotype due to the absence of active repression. This hypothesis is confirmed further by the congenital hypothyroid animal-like cerebellar phenotypes in animals expressing mutated TR that does not bind to thyroid hormones. By comparing several animal models, therefore, the physiological roles of thyroid hormone in brain can be studied further.

Thyroid hormone regulates the expression of most target genes in the brain only at a limited critical period during development. The molecular mechanisms generating such critical period have not yet been clarified. Because the development of rodent cerebellum occurs mostly postnatally and because the anatomical structure of the cerebellum is well characterized, the cerebellum can be a very good system to study such mechanisms. However, cerebellar phenotypes of thyroid hormone system-related animal models have not always been examined, probably because some researchers who generated animals may not be interested in the brain. Further studies are thus required to examine the change in cerebellar structure and gene expression in animal models discussed in this article.

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